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Original Research Article

Root Dentin Surface Roughness and Microhardness Influenced by Different Irrigation Regimens

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Abstract: Endodontic irrigants could alter the mechanical properties of root canal dentin. The aim of the current study was to compare the effect of three irrigation protocols on root canal dentin microhardness and roughness with respect to their concentration and additional ultrasonic activation for the same treatment period. Forty single-rooted, intact upper incisors were decoronated and sectioned longitudinally in buccolingual direction. The surfaces of all eighty samples were polished and randomly allocated into four equal groups (n=20): Group 1: Control group (distilled water); Group 2: 2% NaOCl/2 min followed by 17% EDTA/2 min; Group 3: 2% NaOCl/2 min followed by 17% EDTA/2 min, both ultrasonically activated Group 4: 5.25% NaOCl/2 min followed by 17% EDTA/2 min. Root dentin microhardness was significantly lowered by all of the irrigants regardless of their concentration and/or additional agitation ($p<0.05$). The surface of root canal dentin was roughned to the greatest extent by the ultrasonically activated irrigation solutions ($p<0.001$). The ultrasonic activation of the disinfection solutions significantly altered the biomechanical properties of dentin.

Keywords: Dentin microhardness, dentin roughness, EDTA, irrigation, sodium hypochlorite, ultrasonic activation.

INTRODUCTION

The main goal of the root canal therapy is to increase the long-term survival of non-vital teeth by ensuring thorough disinfection, shaping, hermetic sealing of the root canal system and reliable postendodontic restoration of the affected teeth (Peters, 2004; Tsenova *et al.*, 2018; Hülsmann *et al.*, 2005). The shaping of the root canals causes the formation of a thin, loose layer, comprised of organic and inorganic comopounds, called smear layer (Rapgay *et al.*, 2018; Tsenova-Ilieva & Karova, 2021). The preservation of the latter might compromise the proper sealing of the root canal, and increase the risk of secondary infection (Machado *et al.*, 2021). Endodontic irrigants ensure lubrication, debridement, and dissolution of the smear layer components. Additionally, they should act antimicrobially towards numerous pathogenic bacteria and their toxins. To date not a single irrigant is able to fulfil all these requirements (Tsenova *et al.*, 2018; Duvvi *et al.*, 2018; Tsenova-Ilieva & Karova, 2020a).

Smear layer can be reliably removed by the combined action of endodontic files and different irrigants. Nevertheless, the disinfection solutions can influence the mechanical properties of dentin such as its microhardness, solubility, permeability and surface roughness (Sayin *et al.*, 2007; Doğan & Qalt, 2001; Ari & Erdemir, 2005). Several studies found that these effects are related with the concentration and the time of the application of the irrigants (Saleh, 2016; Bakr *et al.*, 2016; Sayin *et al.*, 2009).

The most widely used endodontic irrigants are sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA). NaOCl affects the organic part of the smear layer, whereas EDTA chelates the mineralized one (Hülsmann, 2013; Zehnder, 2006; Basrani & Haapasalo, 2012). Literature data are inconclusive regarding the application time, concentration, ultrasonic activation and the sequence in which these irrigants are utilized without hampering the biomechanical properties of dentin (Tsenova-Ilieva & Karova, 2021; Tsenova-Ilieva & Karova, 2020a).

Microhardness testing enables a comprehensive, non-destructive, easily performed

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investigation of the fine scale changes in the microhardness of root dentin (Tsenova-Ilieva & Karova, 2020a; Saha *et al.*, 2017; Abbas *et al.*, 2018). It is described as the resistance to local deformation measured on the basis of the induced permanent surface deformation that remains after removal of the load (Abbas *et al.*, 2018). Although this *in vitro* test is not clinically applicable, the determination of the hardness profile can be considered as an indirect proof of the mineral constitution of dental hard tissues (Das *et al.*, 2014; Ari *et al.*, 2004). Such changes may have detrimental effect over the adhesive properties of root canal dentin and decrease the root strength, thus increasing its fracture susceptibility (Tsenova-Ilieva & Karova, 2020a; Bakr *et al.*, 2016; Cruz-Filho *et al.*, 2011).

Atomic force microscopy (AFM) has been advocated as a non-destructive imaging technique in various scientific disciplines (Tsenova-Ilieva & Karova, 2020b). This method could map the sample topography by following an atomic-force field on a surface in a non-destructive manner (Kubinek *et al.*, 2007; Silikas *et al.*, 2001). It offers a comprehensive three-dimensional image of the surface topography of an object in a nanometric resolution (Kubinek *et al.*, 2007).

The aim of the current study was to assess the effect of three endodontic irrigation protocols on root canal dentin microhardness and roughness. The null hypothesis was that all of the disinfection solutions would act similarly regardless of their concentration and ultrasonic agitation at one and the same application period.

MATERIAL AND METHODS

Teeth selection and preparation

The experiment was approved by the Research Ethics Committee of Medical University – Sofia, Sofia, Bulgaria (№838/05.03.2020). A total number of forty upper central incisors from patients were obtained from a pool of recently extracted due to periodontal lesions teeth from the Department of Oral and Maxillofacial Surgery, Faculty of Dental Medicine, Medical University – Sofia, Bulgaria. All patients, aged 40-55 years, signed informed consent. The teeth were initially immersed in water solution of 0.1% thymol at 37 °C. The external surface of all samples was cleaned from calculus and plaque using hand periodontal curettes and stored in distilled water until further use. The selection of teeth was based on their relative dimensions and morphological similarities, verified by means of X-rays in different projections. Afterwards, the teeth were examined under a stereomicroscope Leica S6 *(Leica Microsystems, Wetzlar, Germany*) at x20 and x40 magnification for detection of external root damage. All incisors having any root or coronal defects such as caries and cracks, apical curvature more than 5°, fractured and/or immature root apices, calcifications and resorption were eliminated from the investigation and replaced with new freshly extracted teeth.

The samples were not endodontically treated. The crowns of all teeth were removed at the level of the cementoenamel junction with a diamond disk under copious water irrigation. The roots were approximately 16 mm in length. The roots were further sectioned longitudinally buccolingually by a saw microtome Leica SP1600 *(Leica Microsystems, Wetzlar, Germany*) under copious water cooling and a total number of 80 samples was obtained. Root specimens were horizontally positioned into acrylic resin moulds with the dentin surface pointing upwards. Each root surface was polished with a sequence of increasingly finer polishing discs OptiDisc (*Kerr Dental, Orange, CA, USA*) in the following order: Extra-Coarse (80µm), Coarse/Medium (40µm), Fine (20µm), Extra-Fine (10µm) under copious water coolant and the integrity of the dentinal surface was stereomicroscopically assessed. The prepared samples were randomly allocated into four experimental groups (n=20) and subjected to the following irrigation regimens, where each solution was used in a total amount of 50 ml for a period of 2 minutes:

Group 1: distilled water (control group).

Group 2: 2% sodium hypochlorite (NaOCl) followed by 17% EDTA.

Group 3: 2% sodium hypochlorite (NaOCl) followed by 17% EDTA (both solutions were agitated ultrasonically).

Group 4: 5.25% sodium hypochlorite (NaOCl) followed by 17% EDTA

Exposed root canal dentinal surfaces of each root half were immersed in glass plates containing 50 ml of the disinfection solutions at a room temperature for 2 miniutes. The irrigants were replaced with new ones after the treatment time of each sample. Immediately after the action of each of the irrigant solutions all specimens were rinsed with 50 ml distilled water to eliminate the possible chemical reaction between them and the prolonged chelating effect of EDTA. The passive ultrasonic activation in group 3 was accomplished with the aid of a Digital Ultrasonic Cleaner CD-4820 (*Shenzhen Codyson Electrical Co., Ltd, China*) at 42,000 Hz frequency.

After the action of the disinfection solutions half of the samples in each group underwent microhardness testing, while the rest of the specimens were used to measure the dentin roughness by means of atomic force microscopy.

Microhardness testing

The samples were left to dry on absorbent paper and then mounted on the stage of Micro Vickers & Knoop Hardness Tester (*Indentec Hardness Testing Machines Limited, United Kingdom*). Each root half was divided into three segments – coronal, middle and apical. Two indentations were made on the dentinal surface on both sides of the canal lumen in the three tested areas of each sample. Each measurement was carried out using a 300g load with a dwell time of 20 seconds, applied at approximately 500µm distance from the pulp-dentin interface, oriented perpendicularly to the root surface. The diagonal lengths of the indentations were calculated by a built in software and automatically converted into Vickers hardness numbers (VHN) displayed on the monitor of the device. All six values were averaged to produce the final microhardness value of each specimen.

Atomic force microscopy

The surface roughness of all specimens was determined by using an atomic force microscope MultiMode V (*Veeco Instruments Inc.*) and Controller NanoScope V (*Bruker Ltd, Germany*). The testing was performed in four areas of the midroot region of each segment by soft tapping mode AFM probes (*Tap 150Al-G, Budget Sensors, Innovative solutions Ltd., Bulgaria*) with aluminum reflective coating. A dynamic, tapping mode, in an ambient experimental environment, at a scanning speed of 0,5 Hz and scanning size of 10 µm x 10 µm was applied. All images were obtained in a topographic scanning mode, 512 x 512 pixels in JPG. format and further analysed by NanoScope software.

The overall roughness of the dentin surface was measured in nanometers by the Ra parameter (the arithmetical average value of all absolute distances of the roughness profile from the centreline within the measuring length). The arithmetical mean roughness value (Ra, nm) for each root half was estimated and averaged to produce the final roughness value of each specimen.

Statistical Analysis

Results were analysed with the IBM SPSS Statistics 23.0 software (*International Business Machines Corporation, New York, NY, USA*). Mean and standard deviations (SD) were calculated for all the variables. To check the normality of the data distribution Kolmogorov–Smirnov test was utilized. Statistical analysis for intergroup comparison of dentin microhardness was carried out using one-way ANOVA. Post-hoc Tuckey test was run to find out the pair-wise mean microhardness difference between two groups with 95% confidence level. Statistical analysis for intergroup comparison of the values of Ra was done by using the Kruskal-Wallis H test. Post-hoc Dunn-Bonferroni test was carried out to establish the pair-wise mean roughness difference between two groups. The confidence level was set at 95% ($p<0.05$).

Results

Vickers microhardness and Ra values (mean ± SD) for the irrigating protocols are summarized in table 1. Vickers hardness number data was normally distributed (Kolmogorov–Smirnov test). One-way ANOVA demonstrated that the mean hardness values among the four groups after the specimens were immersed in their respective irrigants differ statistically ($p<0.05$). The subsequent post-hoc Tuckey test showed that the three experimental groups reduced the mean hardness of dentin significantly when compared to the control group ($p<0.001$). Pair-wise comparison between the values of the experimental groups exhibited no statistical significance ($p>0.05$) regardless of the different mode of irrigation (Table 1).

All tested irrigation protocols increased root dentin surface roughness in comparison with the control group (Group 1) (Figure 1). Kolmogorov–Smirnov test showed that the data was not normally distributed. The analysis of the surface roughness values (Kruskal-Wallis H test) showed significant differences between the Ra values in the experimental groups ($p<0.05$) (Table 1). The post-hoc test for the intergroup comparison revealed that the root dentin surfaces were significantly rougher in the group where additional activation was used (Group 3) in comparison with those in the other experimental groups.

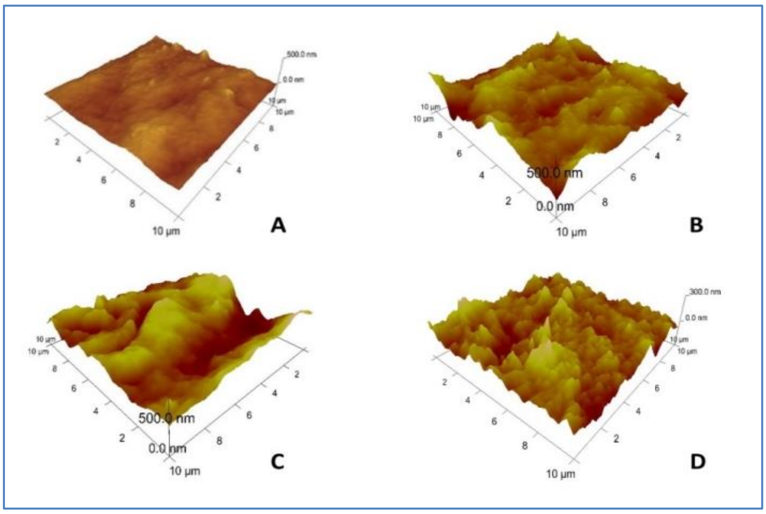

Fig-1: Surface roughness reconstructions of the scanned areas of root canal dentin: A) Control group, B) Group 2, C) Group 3, D) Group 4.

Table-1: Comparison of the microhardness and roughness values in each group.

Group	n	Irrigation regimen	Microhardness (VHN)	Roughness (Ra, nm)
1	10	Control group	60.43 ± 3.34^{a}	52.50 ± 4.22^{a}
2	10	2% NaOCl/2 min + 17% EDTA/2 min	51.77 ± 6.15^{b}	54.26 ± 4.54^{a}
3	10	2% NaOCl/2 min/ US + 17% EDTA/2 min/US	50.67 ± 5.15^{b}	147.38 ± 56.78^{b}
4	10	5.25% NaOCl/2 min+ 17% EDTA/2 min	50.90 ± 2.84^{b}	56.60 ± 5.12^{a}

* Statistical significance is labelled with different superscript letters.

DISCUSSION

All tested irrigation regimens in the present study affected the root dentin biomechanical properties compared to the control group, thus the null hypothesis was rejected. Our findings corroborate with previous reports that disinfection solutions used in the course of the endodontic therapy might alter the microhardness and roughness of root canal dentin (Saleh, 2016; Ballal *et al.*, 2010; Hu *et al.*, 2010; Ballal *et al.*, 2015; Farshad *et al.*, 2017; Kumar & Anita, 2014; Patil Uppin, 2011; Quiteifan *et al.*, 2019).

Human root dentin is a non-homogenous biological material (Duvvi *et al.*, 2018; Tsenova-Ilieva & Karova, 2020a). Tartari et al. concluded that despite being structurally different dentin surface in the root thirds behaves similarly when subjected to one and the same irrigation regimen (Tartari *et al.*, 2013a). Based on this presumption, we calculated and analyzed the mean hardness and roughness values for each sample. *Pashley et al*. (1985) reported that dentin hardness depends on the location and its value decreases when the indentations were made in close proximity to the pulp (Pashley & Parham, 1985). For better standardization of the experimental conditions in all groups the microhardness indentations were executed at 500 µm distance from the canal lumen of each root half (Abbas *et al.*, 2018; Baldasso *et al.*, 2017). The application of 300g load for 20 sec dwelling time was in line with with earlier microhardness studies (Duvvi *et al.*, 2018; Saleh, 2016). The middle area of the root was used for the atomic force microscopy, in the region halfway between the root canal and cementum, since at this site the dentinal tubules are evenly distrubited and exhibit almost the same size (Patil & Uppin, 2011).

In an attempt to ensure a reproducible and unbiased experimental design, all of the roots were sectioned longitudinally into two halves which is in line with previous investigations (Tsenova-Ilieva & Karova, 2020a; Ballal *et al.*, 2010; Hu *et al.*, 2010; Ballal *et al.*, 2015; Farshad *et al.*, 2017; Patil & Uppin, 2011; Öztekin & Adıgüzel, 2019). According to Cruz-Filho et al. this way of sample preparation resembles the clinical situation more accurately (Cruz-Filho *et al.*, 2011). We intended to provide a plane for measurement relatively close to the original morphology of the dentinal surface and assess the sole effect of the irrigation solutions on the biomechanical properties of dentin, thus the root canals were not mechanically instrumented prior the testing (Duvvi *et al.*, 2018; Saleh, 2016; Bakr *et al.*, 2016; Quiteifan *et al.*, 2019; Akcay *et al.*, 2013).

Literature is inconslusive regarding the application time and concentration of the endodontic solutions necessary for an efficient and safe smear layer removal without impairing the mechanical properties of dentin tissue (Tsenova-Ilieva & Karova, 2020a). Our results showed that even a short treatment period of 2 minutes per irrigant with or without ultrasonic activation significantly decreased the root dentin microhardness compared to the control group. These findings support the conclusions of Akcay *et al*. (2013) who concluded that the successive irrigation with 7.5% EDTA followed by 2.5% NaOCl for one minute decreased the dentin microhardness in comparison with its initial value (Akcay *et al.*, 2013).

The scientific data concerning the successive action of NaOCl and EDTA on dentin microhardness and roughness is insufficient. Niu *et al*. (2002) observed dentin erosion when EDTA is followed by NaOCl irrigation. The demineralization of dentinal tubules leads to the enlargement of their orifice diameters and lowers the intertubular dentin thickness. This might alter the viscoelastic properties of dentin (Niu *et al.*, 2002). In order to avoid aggressive erosion, we immersed the specimens in NaOCl solution only once, before the application of EDTA (Saleh, 2016).

The separate use of 2.5%, 5.25% NaOCl, and 17% EDTA solutions increased the surface roughness of the root dentin (Ari *et al.*, 2004; Hu *et al.*, 2010; Ballal *et al.*, 2015; Ratih *et al.*, 2020). Tartari *et al*. studied the effect of the sole use of irrigants and reported that the increase of root dentin surface roughness was only attributed to the chelators. Moreover, they concluded that the single use of 2.5% NaOCl and its utilization before or after chelating solutions did not affect the surface topography of dentin (Tartari *et al.*, 2013a). The same conclusions were made by Akbulut *et al*. (2019) in their investigation about the effect of different fruit vinegars (Akbulut *et al.*, 2019). Keine *et al*. (2020) reported that the combined action of NaOCl and EDTA roughened the radicular dentin to the greatest extent when compared to the saline group (Keine *et al.*, 2020). We observed that the examined concentrations of sodium hypochlorite followed by the immersion of 17% EDTA increased surface roughness, though the difference between them were not statistically significant.

Passive ultrasonic activation has been advocated as a method for enhanced smear layer removal (Plotino *et al.*, 2007). It might be speculated that the acoustic streaming of the disinfecting solutions could have adverse effect over the biomechanical characteristics of root dentin. The analysis of the data revealed that the groups with the ultrasonic agitation significantly increased root dentin roughness and lowered its microhardness in comparison with the control group. Nevertheless, all the irrigants significantly reduced the Vickers hardness number values, regardless of the irrigation mode.

There are several limitations of the current investigation. Experimental conditions differ considerably from the clinical situation in terms of the amount and the way the irrigation and the activation was performed. However, through the immersing approach a large amount of disinfection solutions is evenly distributed and is in close proximity with the exposed dentin surface (Tsenova-Ilieva & Karova, 2021).

Although the selected methodologies could provide repeated testing without damaging the samples, we have used different specimens to register the changes in the biomechanical characteristics of dentin – microhardness and roughness. Another deficiency of the study is the absence of baseline values in both of the experimental methods. Instead, we utilized a negative control group where all specimens were immersed in distilled water (Saleh, 2016; Quiteifan *et al.*, 2019; Tartari *et al.*, 2013b).

Further experiments should be conducted to assess the effect of various irrigation protocols on radicular dentin microhardness and roughness in a close-end root canal space with or without additional ultrasonic activation (Duvvi *et al.*, 2018).

CONCLUSIONS

Within the limitations of the current *in vitro* investigation, it can be concluded that all tested irrigants significantly lowered the microhardness of root dentin and increased its roughness. The root dentin surface significantly roughenes in case of ultrasonic activation of the disinfection solutions.

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